Title: "Contextualizing action observation in the predictive brain: Causal contributions of prefrontal and middle temporal areas"

Running title: Neural underpinnings of contextualized action prediction

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Abstract

Context facilitates the recognition of forthcoming actions by pointing to which intention is likely to drive them. This intention is thought to be estimated in a ventral pathway linking MTG with frontal regions and to further impact on the implementation of sensory predictions within the action observation network (AON). Additionally, when conflicting intentions are estimated from context, the DLPFC may bias action selection. However, direct evidence for the contribution of these areas to context-embedded action representations in the AON is still lacking. Here, we used a perturb-and-measure TMS-approach to disrupt neural activity, separately in MTG and DLPFC and subsequently measure cortico-spinal excitability while observing actions embedded in congruent, incongruent or ambiguous contexts. Context congruency was manipulated in terms of compatibility between observed kinematics and the action goal suggested by the ensemble of objects depicted in the environment. In the control session (vertex), we found an early facilitation and later inhibition for kinematics embedded in congruent and incongruent contexts, respectively. MTG stimulation altered the differential modulation of M1 response to congruent vs. incongruent contexts, suggesting this area specifies prior representations about appropriate object graspability. Interestingly, all effects were abolished after DLPFC stimulation highlighting its critical role in broader contextual modulation of the AON activity.

1. Introduction

Observing other people´s actions involves the activation of a set of frontal, parietal, and temporal areas collectively termed the Action Observation Network (AON), which are thought to underpin our ability to perceive and comprehend others' behaviors (Caspers, Zilles, Laird, & Eickhoff, 2010; Grafton, 2009). Evidence from neuroimaging studies (Buccino et al., 2004; Calvo-Merino, Glaser, Grezes, Passingham, & Haggard, 2005) shows that activity within this network is modulated by the observer's ability to perform the movements. Furthermore, transcranial magnetic stimulation (TMS) studies show that this activity replicates the muscular involvement (Alaerts, Swinnen, & Wenderoth, 2009; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006) and temporal profile of the observed action (Borroni, Montagna, Cerri, & Baldissera, 2005; Gangitano, Mottaghy, & Pascual-Leone, 2001; Urgesi et al., 2010), thus pointing to the existence of a motor resonance mechanism that maps observed movements onto one's own action representations. Yet, at a mechanistic level this proposal is not trivial: how do we understand others' intentions by simply observing their movements? Originally developed in the domain of basic visual perception, predictive coding framework provides an explanation to this controversial question (Kilner, Friston, & Frith, 2007).

The core proposal of predictive coding in the action domain is that forward models, which are used in action execution to predict the expected sensory consequences of our own movements (Wolpert & Flanagan, 2001; Wolpert & Miall, 1996), can be inverted and used to infer other people's actions. This inferential processing relies on a hierarchical action representation architecture (Grafton & Hamilton, 2007) that involves different levels: (i) muscle, which codes for the pattern of muscular activity required to execute the action (e.g., activation of flexion-extension synergies); (ii) kinematics, which maps the movements of the effectors in space and time (e.g., precision vs. whole hand grasping); (iii) goal, which includes the short-term transitive or intransitive aim (e.g., bringing

an object toward vs away from the body); and (iv) intention, which includes the long-term purpose behind the action (e.g., eating vs. offering). Thus, given the observer's prior about other's likely intention/goal, the AON predicts the more concrete aspects levels of action representation (such as motor commands and perceptual kinematics) on the basis of the observer's own motor representations. If the comparison between the predicted and the observed sensory information mismatches, a prediction error is generated and used to update action representation at the different levels of the cortical hierarchy. By reducing the prediction error at all levels of action representation, the most likely cause of the action can be inferred. Although this model offers an elegant explanation of how actions are recognized, it creates a new difficulty: where is the prior representation of others' intention generated?

While previous brain stimulation studies have mainly assessed the functional relevance of the AON to action perception (Pobric & Hamilton, 2006; Tidoni, Borgomaneri, di Pellegrino, & Avenanti, 2013; Valchev, Tidoni, Hamilton, Gazzola, & Avenanti, 2017; Avenanti, Candidi & Urgesi, 2013), evidence on how the AON interacts with higher-order areas is scanty. It has been proposed (Kilner, 2011) that others' intentions are inferred from the context along a ventral pathway (in brain areas outside of the AON) linking the posterior middle temporal gyrus (pMTG) with the more anterior regions of the inferior frontal gyrus. Briefly, this pathway would be involved in retrieving semantic information related to the observed object and associated actions, as well as in the selection, given the intention, of the more likely action representation. The selected representation would then impact on the classical dorsal AON responsible for the encoding of the concrete motor parameters about upcoming movements.

In a series of previous studies (Amoruso, Finisguerra, & Urgesi, 2016; Amoruso & Urgesi, 2016), we combined single-pulse TMS and motor-evoked potentials (MEPs) recording to explore whether top-down contextual information was capable of modulating action coding at lower levels of representation (i.e., muscle and kinematics). We recorded MEPs from the FDI and a control muscle

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while participants watched videos depicting everyday actions embedded in congruent, incongruent or ambiguous contexts. Videos were interrupted before action ending and participants were requested to predict action unfolding. Context-action congruency was manipulated in terms of compatibility between observed grasping kinematics and the setting in which the action was observed. For instance, one of these settings depicted a mug full of coffee and a plate with some biscuits (breakfast scenario). If the observed model grasped the mug by its handle with a precision grip, this condition was coded as congruent. However, if the model grasped the mug using a whole-hand grip from the top, this was coded as incongruent, given that this type of grasping prevented the model from drinking in a context were the highly expected action was "to drink". After the video, two possible descriptors (i.e., to drink and to move) were presented and participants had to select which was the actor's more likely intention, given contextual and kinematics information present in the video. In addition, we used ambiguous contexts where both type of actions and associated grasping movements were equally plausible (i.e., a mug half full of coffee). We found that, as compared to the ambiguous condition, congruence between the observed movements and the contextual setting facilitated the motor cortex at early stages (~240ms after action onset), while incongruence between them resulted into a later inhibition (~400ms after action onset). Overall, these results were interpreted as reflecting predictive processing in M1, triggered by areas outside the AON. We reasoned that this paradigm suited well for testing where context-based priors might be generated since it allows the manipulation of contextual information in terms of its compatibility with the forthcoming movements. Furthermore, the different timing and mechanisms of the observed effects (i.e., early facilitation and later inhibition) suggested that they might reflect signals connecting the AON with two different pathways. The first pathway, likely involving pMTG (Kilner, 2011), may mediate the generation of context-based expectations and lead to the congruence facilitation effect at an early time window, namely around 240ms, when muscle-specific motor resonance responses are observable in M1 (Barchiesi & Cattaneo, 2013; Naish, Houston-Price, Bremner, & Holmes, 2014). The other pathway may be involved in detecting

interference and inhibiting disconfirmed action representations in M1 based on contextual information. A likely candidate involved in this later pathway is the prefrontal-premotor route. Evidence from primates studies (Cai & Padoa-Schioppa, 2014; Saleem, Miller, & Price, 2014; Takahara et al., 2012; Tsujimoto, Genovesio, & Wise, 2011) suggests that top-down signals from the dorsolateral prefrontal cortex (DLPFC) bias action selection in premotor regions. In a similar vein, action selection theories suggest that, when alternative representations compete for further processing in the AON, the DLPFC biases action selection towards context-relevant information (Cisek, 2006, 2007). Furthermore, there is evidence that the DLPFC might be specifically involved in providing top-down signal when anomalies in action representation within a semantic context are detected (Balconi & Vitaloni, 2012, 2014). Thus, the DLPFC seems to be the best candidate for mediating the late inhibition of motor facilitation when actions are observed in incongruent contexts (Amoruso et al., 2016).

However, it is worth mentioning that recent studies provide evidence for the involvement of the DLPFC in the early generation of top-down predictions about object's identity (Calderone et al., 2013; Kveraga, Boshyan, & Bar, 2007), motion direction (Rahnev, Lau, & de Lange, 2011) and, interestingly, context-based action recognition (Maranesi, Livi, Fogassi, Rizzolatti, & Bonini, 2014). Thus, an alternative possibility is that the DLPFC would play a more general and pervasive role in building-up context-based predictions of others' actions rather than being only recruited in the presence of semantic anomalies.

Here, we aimed to study the functional contribution of two brain nodes beyond the AON in the motor representation of context-embedded actions. Specifically, we tested the involvement of left pMTG and DLPFC in the generation of context-based expectations and in the inhibition of conflicting action representations, respectively. We used a perturb-and-measure transcranial magnetic stimulation (TMS) approach, which offers the unique possibility to i) transiently disrupt neural activity in regions of interest using off-line continuous theta burst stimulation (cTBS) and to

ii) measure the consequent functional modulation of corticospinal excitability (CSE) to observed actions via online single-pulse TMS of M1. This approach, originally developed by Avenanti, Bolognini, Maravita, and Aglioti (2007) has been used in various previous studies (Arfeller et al., 2013; Avenanti, Annella, Candidi, Urgesi, & Aglioti, 2013; Enticott et al., 2012; Ubaldi, Barchiesi, & Cattaneo, 2015; Valchev, Gazzola, Avenanti, & Keysers, 2016) in order to assess the role of brain areas belonging to the classical AON and their contribution to motor resonance. To the best of our knowledge, this is the first study in using this approach to test the involvement of brain areas beyond the AON (MTG and DLPFC) and examine their potential contribution to early/late context-dependent motor resonance responses.

We hypothesized that, if the pMTG (Kilner, 2011) and/or DLPFC (Maranesi et al., 2014) are involved in the generation of context-based expectations about others' intentions during action observation, then, by interfering with its activity, both the facilitation and inhibition of CSE for congruent and incongruent contexts, respectively, should be abolished (H1). In a similar vein, if the DLPFC is involved in solving conflicts between action representations (Balconi & Vitaloni, 2012, 2014), only the inhibitory effect previously observed for incongruent contexts, but not the facilitatory one for congruent contexts, should be disrupted when altering its activity (H2).

2. Materials and Methods

2.1. Participants

A total of eighteen participants (10 women; M = 22.11, SD = 2.89) recruited at the University of Udine took part in the study. One male participant was removed from the analysis due to technical problems during data acquisition, thus all analyses were carried out in a sample of seventeen subjects. Participants were all right-handed according to the Standard Handedness Inventory (Briggs & Nebes, 1975), had normal acuity in both eyes and were free from any contraindication to

TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). They gave written informed consent prior to experimentation and received course credits for participation in the study. The experimental procedures were approved by the local Ethics Committee (Comitato Etico Regionale Unico, Friuli Venezia Giulia, Italy) and were carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. None of the participants reported history of neurological, psychiatric, or other major medical problems. No discomfort or adverse effects during TMS and MEP acquisitions were reported or noticed. All participants were naïve to the purpose of the experiment and a detailed debriefing was provided only after the whole experiment was completed.

2.2. Visual stimuli

The video-clips were recorded in color at 30 frames per second with a Canon EOS 550D camera and were further edited with the Adobe Premiere Pro CS3 3.0 software. Length was matched across videos so that they had an equivalent duration (15 frames for a total of 500 ms). All videos depicted a female model performing reach-to-grasp movement of different objects (i.e., a mug, a glass, a hammer) with her right hand. Depending on the kinematics (precision vs. whole-hand grips), each object could be grasped to perform either one of two possible actions. For instance, in the case of the object "mug", the two possible actions were a) to drink and b) to clean, each of them performed with the correspondent kinematics: reaching-to-grasp and drinking using a precision grip, and reaching-to-grasp and lifting using a whole-hand grip (Iacoboni et al., 2005). See Figure 1.

Actions were recorded in three different contextual settings: congruent, incongruent, and ambiguous. In the congruent condition, the action suggested by the context was compatible with the action suggested by the movement kinematics (i.e., reaching-to-grasp a mug full of coffee with a precision grip). Conversely, in the incongruent condition, the context interfered with the perception of the movement kinematics by cueing to the opposite action (i.e., reaching-to-grasp an empty mug with a precision grip). Finally, in the ambiguous condition, both actions were equally facilitated by the contextual constraints (i.e., the mug was half-full of coffee). For a complete description of

objects, action labels, grip types, contexts, and their possible combinations, please refer to Amoruso and Urgesi (2016).

Stimuli were validated in a previous study (Amoruso & Urgesi, 2016), which confirmed the appropriate manipulation of the plausibility of each action when embedded in congruent, ambiguous, and incongruent contexts (respectively, with parametrically decreasing levels of plausibility). Furthermore, to ensure that the observed CSE modulations were actually due to the manipulation of the contextual information and not to possible subtle differences in the kinematic profiles of the same action across scenes, we performed a frame-by-frame kinematic analysis of the visual stimuli. The analysis showed that movement kinematics (i.e., index finger flexion and wrist flexion angles) for each particular action was not significantly different across congruent, incongruent and ambiguous contexts (Amoruso et al., 2016).

2.3. Action Prediction Task

In a two-alternative forced choice (2AFC) task, participants were requested to watch the video-clips and predict the unfolding of the observed movement kinematics. A temporal occlusion paradigm was used, with the video-clip being stopped two frames before the model made contact with the object. Note that participants observed the pre-shaping of the hand configuration during the reaching-to-grasp phase of the movement and not the grasping movement itself. To build-up their predictions, participants were instructed to carefully pay attention to both aspects of the scene: the kinematic information of the model's hand movements and the contextual information, in terms of objects configurations, in which these movements were embedded. The inclusion of ambiguous trials, in which context was not informative on how the observed action would unfold, prevented participants from focusing their attention only on the contextual cues when responding. Together, our task enabled us to manipulate: i) *context informativeness*: in terms of an overall distinction between contexts providing (congruent and incongruent) or not providing (ambiguous) information

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about action unfolding; and ii) *context congruency*: in terms of the compatibility (congruent) or incompatibility (incongruent) between the actions predicted by the context and observed movement kinematics.

2.4. General Experimental Design

Overall, the experiment included 3 spTMS "measure" sessions in which motor-evoked potentials (MEPs) were recorded simultaneously from the First Dorsal Interosseous (FDI) and from the Extensor Carpi Radialis (ECR, control muscle) of the right hand during task performance. Prior to each measure session, participants underwent a "perturb session", where cTBS was administered over the left pMTG, left DLPFC or over vertex (active control site). All sessions took place in the same day, were separated by 60 min (to minimize carryover effect of cTBS across sessions) and their order was counterbalanced across participants. Figure 2 illustrates the general design structure.

Before running each perturb session, baseline CSE was assessed by acquiring 10 MEPs while participants passively watched a fixation cross. Another series of 10 MEPs were recorded at the end of each measure session.

2.5. Electromyography (EMG) Recording and spTMS

SpTMS was applied to the left M1 using a Magstim 200 stimulator (maximum output = 2 T at coil surface, pulse duration = 250 μsec, rise time = 60 μsec, The Magstim Company, Carmarthenshire, Wales, UK) connected to a 70-mm figure-of-eight coil (Magstim polyurethane-coated coil). Motor-evoked potentials (MEPs) were recorded simultaneously from the right FDI and ECR muscles. Muscle selection was based on previous findings using the current paradigm (Amoruso and Urgesi, 2016; Amoruso et al., 2016) and evidence from spTMS studies showing that while both the FDI and the ECR muscles are activated during the execution of reach-to-grasp movements (Finisguerra, Amoruso, Makris, & Urgesi, 2018), only the FDI corticospinal representation is differently modulated by the observation of a precision vs. whole-hand grip (Urgesi, Candidi, et al., 2006;

Urgesi, Moro, et al., 2006). Thus, based on these findings, we expected a more specific contextual modulation of the FDI muscle as compared to the ECR. Surface Ag/AgCl disposable electrodes (1 cm diameter) were placed in a belly-tendon montage for each muscle. The EMG signal was amplified, filtered (band-pass 5 Hz to 20 kHz) and recorded with Biopac MP-36 system (BIOPAC Systems, Inc., Goleta, CA) at a sampling rate of 50 kHz.

The coil was positioned tangentially on the scalp, with the handle pointing backward and approximately 45° lateral from the midline, perpendicular to the line of the central sulcus (Di Lazzaro et al., 1998). This orientation was chosen based on the finding that the lowest motor threshold is achieved when the induced electric current in the brain is flowing perpendicular to the central sulcus (Brasil-Neto et al., 1992; Mills, Boniface, & Schubert, 1992). The optimal scalp position (OSP) for inducing MEPs in the right FDI and ECR muscles was detected by moving the coil in 1-cm steps over the left M1 and by delivering TMS pulses at constant intensity until the largest MEPs for the FDI were found. Then, the OSP was marked with a pen on a tight-fitting bathing cap worn by participants. The coil was held on the scalp by a coil holder with an articulated arm, and its position with respect to the mark was checked continuously to compensate for small movements of the participants' head during data collection.

During the experimental spTMS sessions, the intensity of magnetic pulses was set at 120% of the individual resting motor threshold (rMT), defined as the minimum intensity of the stimulator output able to produce MEPs with amplitudes of at least 50 μV with 50% probability in higher threshold muscle (Rossini et al. 1994) in 5 out of 10 consecutive pulses. This procedure was used in order to avoid saturation of FDI CSE modulation and possible loss of mirror-like modulation (Loporto et al. 2013) and allowed stable MEP recording from both muscles. The rMT ranged from 40% to 55% (M = 45.8%, SD = 4.6%) of the maximum stimulator output. To ensure that there was no unwanted background EMG activity before the magnetic pulse, the signal from both muscles was continuously verified online by visually monitoring the EMG signal. When voluntary contractions

were detected, participants were encouraged to fully relax their muscles. Furthermore, before starting the spTMS measurement session, EMG signals were sent to loudspeakers to provide participants with an auditory feedback of their muscle activity. MEPs' peak-to-peak amplitudes (in mV) were collected and stored in a computer for off-line analysis.

2.6. Trial Structure

Stimuli were presented using E-prime V2 software (Psychology Software Tools) on a 21 inch CRT monitor (resolution, 1024X768 pixels; refresh frequency, 60 Hz). Participants sat in a comfortable armchair in a dimly lit room ~1 m away from the monitor with prone hands resting on a pillow. They were instructed to pay attention to the displayed stimuli and to avoid moving their right hand. Videos appeared at the center of the screen on a neutral background (subtending ~ 15.96° X 11.97° of visual angle). Trials started with a visual warning cue lasting for 5 s (the Italian word "*attendi*", in English "attention") and it was followed by the video-clip presentation lasting 500 ms. TMS pulses were delivered online at either 300 ms or 500 ms after action onset. After the video clip, a frame was presented with the verbal descriptors of the two possible goals (e.g., "*versare*" and "*spostare*", in English "to pour" and "to place", respectively; one located up and the other located down) written in black on a white background. This frame remained on the screen until a response was recorded. Participants were requested to verbalize their responses (by saying "*su*" or "*giù*", in English "up" or "down", respectively) and the experimenter recorded the answer by pressing a computer key (Fig. 2B). The location of the two descriptors was counterbalanced, ensuring that in half of the trials one of the descriptors was presented up and, in the other half, it was presented down. This procedure enabled us to prevent participants from planning their response in advance on the basis of the descriptors' spatial location. Verbal responses were used to prevent that peripheral muscular contraction artifacts resulting from button press contaminated MEPs. Importantly, verbal responses were required only after the TMS pulse was delivered, thus reducing the possibility of interfering with the hand MEPs (Gentilucci, Bernardis, Crisi, & Dalla Volta, 2006). Thus, our

primary behavioral measure was accuracy of responses, since we could not reliably record reaction times in our TMS set up. The interval between TMS pulses was at least 7,500 ms to avoid cumulative effects of the stimulation. A total of 42 video clips (7 action pairs embedded in 3 different contexts) were created. Specifically, we used 7 different objects that could be reach-to-grasp to perform either one of two possible actions differing in terms of precision vs. whole-hand grips. In addition, each action was presented in congruent, incongruent and ambiguous contexts. Overall, each video was presented twice, for a total of 84 stimuli randomly presented in 2 blocks of 42 trials each.

2.7. cTBS and Neuronavigation

The cTBS protocol lasted 20 s and consisted of trains of 3 bursts of TMS pulses delivered at 50 Hz every 200 ms (at 5 Hz), for a total of 300 pulses, over the target area (Vertex, pMTG or DLPFC) (Oberman, Edwards, Eldaief, & Pascual-Leone, 2011). This protocol is known to reduce the excitability and alter the functions related to the target area for at least 20 min (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). After cTBS, it took no more than 5 min to start the "measure session", which permitted us to capture the effect of the stimulitation when it reached its maximum (Huang et al., 2005).

Stimulation was administered with a 70mm Double Air Film Coil connected to a Magstim Rapid2 stimulator (The Magstim Company, Carmarthenshire, Wales, UK). Stimulation intensity was set at a fixed intensity of 40% of maximum stimulator output (MSO). This fixed intensity was chosen because it corresponded to the rMT of the participant with the lowest threshold and could thus be considered safe for all the participants (Wassermann, 1998).

Coil position was identified on each participant's scalp with the SofTaxic Navigator system (EMS, Italy). Skull landmarks (nasion, inion, and preauricular points) and 65 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra Optical Tracking System (NDI, Canada). Coordinates in Talairach space were automatically estimated by the SofTaxic

Navigator from an MRI-constructed stereotaxic template. In the case of the left pMTG (x = −54, y = -49, and z = -2), coordinates were estimated from the results of previous studies investigating the neuroanatomical underpinnings of semantic knowledge, as highlighted in a meta-analysis of neuroimaging results (Noonan, Jefferies, Visser, & Lambon Ralph, 2013) and, in particular, the correlates of tool naming deficits in brain lesion patients (Campanella, D'Agostini, Skrap, & Shallice, 2010). For the left DLPFC position (x = −52, y = 32, and z = 20), we used the coordinates recently reported in a TMS study investigating the role of top-down prefrontal signals in modulating M1 responses (Ubaldi et al., 2015). As a control site (i.e., to test for nonspecific effects of TMS), the vertex (x = 0, y = −44, z = 69) was stimulated with the induced current running from posterior to anterior along the inter-hemispheric fissure as in a previous study (Cazzato, Mele, & Urgesi, 2014). No adverse effects during cTBS were reported or noticed in any participant.

2.8. Data Analysis

2.8.1.Behavioral Analysis

Individual performance expressed as *d* prime values (*d'*), a bias-corrected measure of sensitivity in discriminating between 2 categories (Macmillan & Kaplan, 1985), was calculated for each experimental condition and session. In the *d'* analysis, 'precision grips' identified as 'precision grips' were considered as "hits", 'whole-hand grips' identified as 'precision grips' were considered as "false alarms", and 'precision grips' identified as 'whole-hand grips' were considered as "missed responses". More specifically, correct responses were defined by the kinematics, not by the context (e.g., when the participant observed a whole hand grip of the bottle in a context where the glass is full, "pour" was the correct answer). The *d'* values were calculated by transforming the response proportion to *z*-scores, and then subtracting the *z*-score that corresponds to the false-alarm rate from the *z*-score that corresponds to the hit rate (Stanislaw & Todorov, 1999). The *d'* values were subjected to a repeated-measures analysis of variance (RM-ANOVA) with session (vertex, pMTG,

DLPFC), time (early, late) and context (congruent, incongruent, ambiguous) as within-subjects variables.

2.8.2. MEP Analysis

Peak-to-peak amplitudes of the MEPs (in mV) from both the FDI and ECR muscles were recorded from individual trials and averaged separately for each condition, muscle and participant. Since background EMG is known to modulate MEP amplitude, it was assessed in each participant by calculating the mean rectified EMG signal across a 100 ms interval prior to TMS. MEPs with preceding background EMG deviating from the mean by >2 SD were removed from further analysis. The total percentage of excluded MEPs (FDI: Vertex = 3.8%; pMTG = 5.6%; DLPFC = 4.9%; ECR: Vertex = 3.2%; pMTG = 5.2%; DLPFC = 4.8%) was not significantly different across sessions and muscles (all Fs<3.11, and all Ps >0.06). Following Osborne (2002) recommendations, raw MEP values were log-transformed to address non-normality resulting from positive skew. After transformation, baseline MEP amplitude values recorded at the beginning and at the end of each session were collapsed and entered into a RM-ANOVA including Session (vertex, pMTG, DLPFC) and Muscle (FDI, ECR) factors.

MEP amplitudes recorded during action observation were expressed as difference values from MEPs recorded during baseline (fixation cross) within each stimulation session. These normalized action-observation MEPs were analyzed by means of a 4-way RM-ANOVA with Session (vertex, pMTG, DLPFC), Muscle (FDI, ECR), Time (early, late) and Context (congruent, incongruent and ambiguous) as within-subjects' variables. Estimates of the effect size were obtained using the partial eta-squared ($\eta_p{}^2$). In all ANOVAs, post-hoc analysis was carried out using the Duncan test correction for multiple comparisons. The α value for all statistical tests was set at 0.05. The analyses were implemented in Statistica software v.10 (Statsoft, Tulsa, OK).

2.8.3. Supplementary Analysis

Vertex stimulation is commonly used in rTMS experiments as an active control stimulation since it is more similar to the active site stimulation in terms of tactile and auditory sensations as compared

to other sham stimulation methods (Sandrini, Umilta, & Rusconi, 2011). However, because of its vicinity to sensorimotor areas interconnected with the AON, we aimed to check that cTBS to vertex did not induce any off-line modulation of CSE during the "measure session". Thus, we conducted a supplementary analysis where we compared the MEP data obtained in the current study (after cTBS to vertex) with the MEP data obtained in a previous study (Amoruso et al., 2016) using the same task and stimuli and similar procedures. More specifically, we ran a RM-ANOVA including muscle (FDI, control forearm), context (Congruent, Incongruent), and time (early, late) as the within-subject factors, and study (cTBS, no-cTBS) as the between-subject one. To ensure comparability across datasets, we log-transformed data from our previous study (Amoruso et al., 2016) and calculated two congruency indexes expressed as the ratio between, on the one hand, congruent and ambiguous conditions (congruent index) and, on the other hand, between incongruent and ambiguous conditions (incongruent index).

3. Results

3.1. Behavioral Results

Participants' sensitivity scores (d') in recognizing the different kinematics' patterns (precision vs whole-hand grips) and predicting action unfolding in each context and session are reported in Table 1. The RM-ANOVA performed on the d values yielded a main effect of Context ($F_{2, 32} = 95.96$, $p<0.001$, $\eta_p^2 = 0.85$) and an interaction between Session, Time and Context ($F_{4,64} = 2.53$, $p = 0.04$, $\eta_p^2 = 0.13$). Post-hoc comparisons on the interaction (MSE = 0.04788, df = 64) indicated that, regardless of the session, participants performed better in predicting the unfolding of the observed actions when they were embedded in congruent contexts as compared to both ambiguous and incongruent ones (all ps < 0.001). In addition, action recognition was better in ambiguous as compared to incongruent contexts (all ps < 0.001). Notably, after cTBS was administered to the left DLPFC, participants' sensitivity in recognizing actions embedded in either congruent (early time window) or incongruent contexts (later time window) was significantly reduced relative to vertex (p

= 0.02, and $p = 0.01$ respectively). This latter difference was also significant when comparing DLPFC and pMTG ($p = 0.005$). See Fig. 3.

3.2. MEP Results

The mean raw MEP amplitudes recorded from the FDI and the ECR muscles in the three sessions in the different conditions (both action observation and baseline trials) are reported in Table 2. The ANOVA performed on the mean log-transformed MEP amplitudes recorded during the baseline blocks before and after cTBS sessions revealed non significant main effects of Time (pre vs post) ($F_{1,16} = 0.405$, $p = 0.533$, $\eta_p^2 = 0.024$), Session ($F_{2,32} = 0.050$, $p = 0.951$, $\eta_p^2 = 0.003$) and Muscle ($F_{1,16} = 2.25$, $p = 0.152$, $\eta_p^2 = 0.123$) as well as non significant interactions between these factors (all Fs<0.208; all Ps>0.812). Together, these results indicate that baseline MEPs recorded from the two muscles across the three stimulation sessions before and after cTBS stimulation were comparable and there were no effects of cTBS on baseline CSE. Thus, the MEP amplitudes recorded in the baseline condition of each session before and after cTBS were collapsed, used to normalize MEPs for the action observation trials pertaining to each particular session (i.e., the baseline recorded in the DLPFC session was used to normalize MEPs recorded during action observation in the DLPFC session).

The omnibus RM-ANOVA performed on the normalized action observation MEPs revealed significant main effects of Muscle ($F_{1,16} = 24.6$, $p < 0.001$, $\eta_p^2 = 0.60$) and Time ($F_{1,16} = 12.66$, $p = 0.002$, $\eta_p^2 = 0.44$), significant interactions between Muscle and Time ($F_{1,16} = 8.83$, $p = 0.008$, $\eta_p^2 = 0.35$), Muscle and Context ($F_{2,32} = 6.43$, $p = 0.004$, $\eta_p^2 = 0.28$), Session, Muscle and Context ($F_{4,64} = 6.68$, $p < 0.001$, $\eta_p^2 = 0.29$), and, importantly, a significant 4-way interaction between Session, Muscle, Time and Context ($F_{4,64} = 2.78$, $p = 0.03$, $\eta_p^2 = 0.14$). To further analyze this latter interaction, 2 follow-up Session x Muscle x Context RM-ANOVAs were carried out separately for each time window.

3.2.1. Early time window analysis (300ms).

The RM-ANOVA performed on the early time window showed a significant main effect of Muscle ($F_{1, 16} = 24.84$, $p < 0.001$, $\eta_p{}^2 = 0.60$), a significant interaction between Muscle and Context ($F_{2,32} = 3.95$, $p = 0.02$, $\eta_p{}^2 = 0.19$), and a significant 3-way interaction between Session, Muscle and Context ($F_{4,64} = 2.52$, $p = 0.04$, $\eta_p{}^2 = 0.13$). Post-hoc analysis on this interaction (MSE = 0.00027, df = 64) showed that, in the vertex session, motor facilitation for the FDI was enhanced for actions observed within a congruent context (M = 0.169, SEM = 0.02) as compared to those observed in ambiguous (M = 0.157 SEM = 0.02, $p = 0.038$) and incongruent contexts (M = 0.153, SEM = 0.02, $p = 0.009$), which in turn did not differ ($p = 0.48$). During the pMTG session, actions embedded in either congruent (M = 0.154, SEM = 0.02) or incongruent (M = 0.150, SEM = 0.02) contexts elicited an enhanced response as compared to those observed in ambiguous ones (M = 0.137, SEM = 0.02; $p = 0.007$ and $p = 0.04$, respectively). Notably, while in the vertex session there was a difference between congruent and incongruent conditions, this difference was disrupted after pMTG stimulation ($p = 0.44$). During the DLPFC session, all the differences between context conditions were abolished (Congruent: M = 0.145, SEM = 0.02; Incongruent: M = 0.146, SEM = 0.02; Ambiguous: M = 0.141, SEM = 0.01; all ps > 0.44).

When comparing conditions across sessions, significant decreases for the ambiguous and the congruent conditions were found after DLPFC ($p = 0.01$ and $p = 0.0002$, respectively) and pMTG cTBS ($p = 0.002$ and $p = 0.01$, respectively) as compared to vertex cTBS. No modulations were observed for the incongruent condition (all ps > 0.23).

Overall, no modulations were observed for the ECR muscle (all ps > 0.05). See Fig. 4A.

3.2.2. Late time window analysis (500ms).

The RM-ANOVA performed on the late time window showed significant main effects of Muscle ($F_{1,16} = 24.02$, $p < 0.001$, $\eta_p{}^2 = 0.6$) and Context ($F_{2,32} = 4.38$, $p = 0.02$, $\eta_p{}^2 = 0.21$) and significant interactions between Session and Context ($F_{4,64} = 2.78$, $p = 0.03$, $\eta_p{}^2 = 0.14$) and between Session, Muscle and Context ($F_{4,64} = 7.1$, $p < 0.001$, $\eta_p{}^2 = 0.30$). The post-hoc analysis on this interaction

(MSE = 0.00043, df = 64) showed, within the vertex session, facilitation for actions embedded in congruent contexts (M = 0.153, SEM = 0.02) as compared to those embedded in ambiguous (M = 0.129, SEM = 0.01; p = 0.003) and incongruent contexts (M = 0.104, SEM = 0.01; p < 0.001); furthermore, it also showed inhibition for incongruent as compared to ambiguous (p = 0.001) contexts. Both effects were abolished during the DLPFC session (all ps > 0.48). In the pMTG session a different pattern was observed, consisting of facilitation for actions embedded in either congruent (M = 0.1394 SEM = 0.024) or incongruent (M = 0.1396 SEM = 0.023) contexts as compared to ambiguous contexts (M = 0.119 SEM = 0.02; both ps = 0.01). No differences were observed between the congruent and the incongruent conditions (p = 0.97).

When comparing the different conditions across sessions, no modulations were observed for the ambiguous context stimuli (all ps > 0.18). However, a decrease for the congruent condition was observed for DLPFC as compared to vertex (p = 0.002). Finally, for the incongruent condition, CSE during vertex session was lower as compared to both DLPFC and pMTG sessions (p = 0.006 and p = 0.0003, respectively), reflecting the absence of the inhibitory incongruence effects after stimulation of both areas. There was no difference between DLPFC and pMTG sessions (p = 0.32).

Again, no modulations were observed for the ECR muscle (all ps > 0.10). See Fig. 4B.

3.2.3. Supplementary results

The RM-ANOVA performed on the data recorded after cTBS over vertex and the data recorded in the non-cTBS condition (obtained from Amoruso et al., 2016), yielded no main effect of study (F = 0.072; p = 0.789) nor interactions including this factor (all Fs<2.797; all Ps>0.10). This finding rules out the possibility that cTBS over vertex might have modulated MEP responses, ensuring that vertex stimulation worked indeed as an active control condition.

4. Discussion

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In the present study, we used cTBS to temporarily alter neural activity within left pMTG and DLPFC and measured consequent CSE modulations in M1 during the observation of actions embedded in naturalistic contexts. In keeping with previous studies (Amoruso et al., 2016; Amoruso & Urgesi, 2016), we found that, as compared to ambiguous context, participants' prediction of the unfolding action goal was improved when action kinematics was embedded in congruent contexts and was hampered when action kinematics was embedded in incongruent ones. This overall pattern of responses remained constant regardless of session, pointing to the integration of both kinematics and contextual cues in prediction performance.

These behavioral results were mirrored, at the neurophysiological level, during the vertex session where we found an early facilitation (~300ms) and a later inhibition (~500) for movement kinematics observed in congruent and incongruent contexts, respectively. The fact that different results were obtained when the single TMS-pulse was delivered at the early and late time windows, even if the participants could see the action for a comparable amount of time, suggests that peripheral effects of TMS might have interfered with viewing the part of videos after the pulse. These effects may include the "clicking" sound of the TMS coil which can produce a phase resetting of occipital alpha oscillations (Romei, Gross, & Thut, 2012; Romei, Murray, Cappe, & Thut, 2009) and the mechanical vibrations passed from the coil to the scalp inducing eye blinks. Hence, it is possible that the responses obtained during the "measure" sessions are likely to reflect the processing of the initial part of videos before the TMS pulse was delivered (300 ms-long and 500 ms-long, respectively).

At the behavioral and neurophysiologic level, pMTG stimulation did not alter action prediction performance nor disrupt (according to H1) the facilitatory effect for congruent contexts as compared to the ambiguous ones, suggesting that processing in this area is non-necessarily required in generating context-based priors about the overarching goal/intention of a given action. Indeed, while the early CSE facilitation in response to congruent and ambiguous contexts was reduced by

pMTG-cTBS as compared to vertex-cTBS, we could still observe, after pMTG-cTBS, higher CSE for congruent than for ambiguous contexts, at both early and late time windows. Surprisingly, we found a facilitation of the incongruent condition as compared to the ambiguous one in both time windows, resulting in the loss of the differential CSE modulation triggered by congruent and incongruent contexts. Thus, pMTG-cTBS hindered the differential modulation obtained for actions occurring in congruent and incongruent contexts. This suggests that, in partial agreement with our first hypothesis (H1), pMTG is involved in processing grasping-related features of how objects need to be manipulated for successful action selection. This graspability knowledge is used to contribute to the predictive system the specification of the expected grasping kinematics, and impacts on appropriate kinematics coding in the AON.

Conversely, after DLPFC stimulation we observed a reduction in participants' sensitivity to predict the unfolding of action kinematics embedded in congruent and incongruent contexts, and both facilitation and inhibition effects were abolished at the neurophysiological level. In fact, stimulation of DLPFC resulted in a complete disruption (i.e., no CSE differences between conditions) of the facilitatory congruency effect in both early and late time windows, and of the inhibitory congruency effect at the later one. This was corroborated by CSE comparisons across sites, which showed that the early CSE response to congruent contexts was lower after DLPFC-cTBS than after vertex-cTBS, whereas the late CSE in response to incongruent contexts was higher after DLPFC-cTBS than after vertex-cTBS. This suggests that DLPFC is not (only) involved in resolving conflicts between competing action representations when interference is detected (H2), but it might play a crucial role in mediating top-down contextual predictions in the AON.

All the CSE modulations occurring in the three sessions specifically involved the FDI muscle, and no modulations were observed for the ECR. While there is evidence that both muscles are synergistically involved in reach-to-grasp movements execution and observation (Finisguerra et al., 2018), it is also true that, within a coordinated pattern of muscular activity, individual muscles can

be activated at different levels and thus, the compromise of each muscle may vary depending on the type of grip. Indeed, there is evidence (Amoruso et al., 2016; Urgesi, Candidi, et al., 2006; Urgesi, Moro, et al., 2006) supporting a greater involvement of the FDI muscle, as compared to the ECR one, during the observation of precision grips. The specificity of cTBS according to context congruency, time window, and stimulation site as well as the absence of any effects of cTBS on baseline CSE rule out spurious effects of cTBS *per se*.

4.1. pMTG is involved in coding objects' appropriate graspability

A recent influential account suggests that the lateral occipitotemporal cortex (LOTC) constitutes a hub region in combining perceptual, semantic and motor knowledge during action representation (Lingnau & Downing, 2015). Mounting evidence from neuroimaging studies hints that the pMTG, a subregion of LOTC, is associated with the perception and use of human tools (Beauchamp & Martin, 2007; Lewis, Brefczynski, Phinney, Janik, & DeYoe, 2005; Martin, Wiggs, Ungerleider, & Haxby, 1996). For instance,Valyear and Culham (2010) found increased activity within this region while participants observed typical as compared to atypical tool-directed grasping actions. Another study (Jastorff, Clavagnier, Gergely, & Orban, 2011) found that this area is also responsive to the physical constraints (i.e., a barrier determining the height of the arm trajectory for reaching an object) in which an action is observed. Interestingly, it has been recently shown that the left pMTG is sensitive to the number of actions an object can evoke, showing increased activity as the number of possible implied actions related to the observed object augments (Schubotz, Wurm, Wittmann, & von Cramon, 2014). Overall, these neuroimaging studies suggest that the pMTG plays a prominent role in integrating the motoric details of the action with stored semantic knowledge about the objects. In fact, objects evoke those actions that we can typically perform with them (i.e., affordance-like representations), thus facilitating action recognition by constraining the number of expectable actions.

Here we found that, after stimulation of the pMTG, both congruent and incongruent contexts boosted motor facilitation responses as compared to ambiguous ones, resulting in an alteration of the specificity of the context congruency effects that were observed in the vertex session. Since congruent and incongruent conditions depicted identical contextual settings and only became congruent or incongruent when being combined with the observed kinematics, it is likely that cTBS of pMTG interfered with the coding of objects' appropriate graspability. Thus, our findings significantly extend previous neuroimaging findings by showing that pMTG plays a causal role in combining action kinematics motoric components (i.e., grasp) and knowledge about the object being used.

While this is in keeping with the predictive coding account on the involvement of the pMTG in providing information related to the observed object and associated actions (Kilner, 2011), the specific pattern of observed modulations raises the interesting possibility that other regions beyond the pMTG could still process, based on contextual information, a prediction signal that reached the AON even if neural activity of pMTG was hampered (as suggested by our current data, one of these regions might be the DLPFC, see below). Indeed, the contextual modulation of motor resonance responses was not completely abolished after interfering with pMTG neural activity, but it changed into an undifferentiated facilitation for both congruent and incongruent contexts. In the absence of any graspability specification of an action, the predictive system is sending to the motor system a prior about the overarching goal of the action (i.e., to pour, to place) that reflects context processing but is void of any specification of what type of grasp is to be expected. Thus, M1 is facilitated whatever grasping kinematics is met in the environment. In the case of the ambiguous contexts, no prediction about the overarching goal of the action can be made; thus M1 facilitation is reduced, independently of whether grasp-related specific information can be processed in pMTG (at sham, after vertex stimulation) or it cannot (after cTBS). In other words, what is reaching M1 after stimulation of pMTG in those cases where context is informative (i.e., congruent and incongruent

conditions) is a high-level prior about the overarching goal of the action estimated from the context, but this prior does not contain information about the appropriate grasp-to-be-used. This may suggest that pMTG is contributing to predict action unfolding by specifying the grasp that is to be expected given a specific overarching goal.

4.2. Left DLPFC mediates top-down contextual predictions in M1

Action selection theories (Cisek, 2006, 2007) propose that DLPFC is called into play to favor appropriate action selection in the AON on the basis of contextual sensory information. There is evidence that, when conflicting action representations compete for selection, top-down suppression of unwanted motor representations is a key component of conflict resolution (Klein-Flugge & Bestmann, 2012; Klein, Petitjean, Olivier, & Duque, 2014). Furthermore, it has been shown that when participants process incongruent object-related actions, perturbation of the left DLPFC alters the ability to analyze semantically anomalous action sequences (Balconi & Vitaloni, 2012, 2014). More specifically, in a series of studies Balconi et al. (2012, 2014) showed that disrupting prefrontal activity with cathodal (inhibitory) transcranial direct current stimulation (tDCS) resulted in reduced behavioral performance and electrophysiological responses to incongruent action stimuli but not to congruent ones. Overall, these findings were interpreted in favor of a specific involvement of the DLPFC in supporting the processing of semantics anomalies during action observation.

In the light of aforementioned studies, we hypothesized that the left DLPFC may be recruited when information between semantic contexts and the motoric components of the action is conflicting; therefore, its stimulation should disrupt only the late CSE inhibitory effect observed for incongruent contexts, but not the facilitation for congruent vs. ambiguous contexts. However, in contrast with H2 and Balconi et al. (2012, 2014)'s results, we found an overall disruption of both the facilitatory and the inhibitory congruency modulations that were observed in the vertex session (Amoruso et al., 2016; Amoruso & Urgesi, 2016). This suggests that DLPFC may play a crucial role in generating

more general top-down context-based priors rather than being specifically involved in the processing of semantics anomalies while observing actions in naturalistic settings.

It has been recently suggested that the prefrontal cortex might be involved in the early generation of top-down predictions during object recognition (Calderone et al., 2013; Kveraga, Boshyan, & Bar, 2007; Rahnev et al., 2011). During the perceptual processing of naturalistic scenes, coarse information regarding the global shape of the stimuli is carried by low spatial frequencies, whereas finer information such as the edges and borders of the object are conveyed by high spatial ones. According to this view, low spatial frequency information rapidly reaches the prefrontal cortex (~130) and creates an "initial guess," namely a prediction about object's identity, that feeds back into the temporal cortex to assist the recognition process (Bar et al., 2006; Kveraga, Ghuman, & Bar, 2007). Interestingly, the DLPFC exhibits preferential activation for low spatial frequency information (Calderone et al., 2013). Another recent study (Rahnev et al., 2011) has shown that, during perceptual decision-making about motion direction, the presence of prior expectations as compared to their absence increases activity in the left DLPFC. While these studies collectively suggest that this region contributes to prior generation during perceptual decision-making, evidence of its specific involvement in the action domain is still sparse.

Interestingly, a recent study in primates (Maranesi et al., 2014) reported that, during action recognition within predictable contexts, two different patterns of discharge were observed in the premotor cortex: a reactive one associated to movement onset after a go-signal, and a predictive one occurring prior to the go-signal. These findings suggest that a sensory-driven representation of the action and a context-based prediction of the same action may be simultaneously recruited during action recognition. According to the authors, while the first one might be generated through the well-known parieto-frontal pathway of the AON, it is likely that the second one, reflecting prediction signals, might reach the premotor cortex through a prefrontal-premotor pathway (Cai & Padoa-Schioppa, 2014; Saleem et al., 2014; Takahara et al., 2012; Tsujimoto et al., 2011). Our

finding that altering DLPFC activity led to a complete disruption of contextual modulatory effects in M1 is in line with this predictive coding view suggesting the involvement of a prefrontal-premotor route in the contextual prediction of others' actions (Maranesi et al., 2014). Furthermore, they extend previous literature by showing that DLPFC is not only involved in generating top-down predictions during object or motion recognition, but also during the observation of actions performed in naturalistic contextual settings.

However, an important consideration is whether the suppression of both contextual effects on CSE following DLPFC stimulation may reflect a general disruption in attentional processing. Previous studies have shown that anatomical lesions (Szczepanski & Knight, 2014) or TMS interference on DLPFC (Johnson, Strafella, & Zatorre, 2007; Wagner, Rihs, Mosimann, Fisch, & Schlaepfer, 2006) might lead to a reduction in attentional processes to relevant stimuli. Although we cannot completely rule out this possibility, the selectivity of the DLPFC effects according to the recording muscle and the observation condition would speak against it. First, irrespectively of session (vertex, pMTG and DLPFC), all the CSE modulations observed in our study were specifically related to the FDI muscle, which is greatly involved in the observation of precision vs. gross grasping movements (Amoruso et al., 2016; Urgesi, Candidi, et al., 2006; Urgesi, Moro, et al., 2006). This can be held as an index of motor resonance rather than of attentional modulation to motor activation (Finisguerra et al., 2015; Naish et al., 2014; Urgesi et al., 2016). Thus, this finding narrow the possibility of explaining our DLPFC data in terms of a global non-specific decrease in attentional resources. Second, at the behavioural level, after cTBS-DLPFC a decrease in performance was observed in the incongruent and the congruent conditions but not in ambiguous ones, as compared to vertex. A decrease in d values means that participants were more biased to context processing (at the expense of kinematics processing), suggesting that DLPFC-cTBS is not affecting visual perception of objects but it is weakening the integration between context and kinematics information. Furthermore, at the motor facilitation level, DLPFC-cTBS affected MEPs recorded in the congruent

and incongruent condition, but not those recorded in the ambiguous one. If is true that attention can have different effects on task with different difficulty levels, it is very unlikely that attentional effects would be the same for the easiest task (i.e., recognizing action unfolding in congruent contexts) as well as in the most difficult (i.e., in incongruent contexts) but not in the intermediate level task (i.e., in ambiguous contexts). In other words, if cTBS-alteration of DLPFC activity in our study was targeting general attentional processes, it should have affected all conditions comparably or, alternatively, it should have shown a modulation of the effects dependent on task difficulty. Finally, modulation of visual attention is more likely to depend on right DLPFC, in line with evidence showing that sustained visual attention is typically associated with a right-hemisphere dominant network rather than a left one (Awh & Jonides, 2001; Cabeza & Nyberg, 2000; Pardo, Fox, & Raichle, 1991). Accordingly, a recent study has shown that attention modulates activation of right-, but not of left-hemisphere areas during perceptual decisions that were based on variable degree of prior knowledge (Sherman, Seth, & Kanai, 2016).

Overall, the differential level of muscle involvement and the condition-specificity of the DLPFC-cTBS effects, as well as previous evidence suggesting that attentional resources are allocated in a right-hemisphere dominant network argue against an attentional account of the observed modulations and speak in favor of a functional involvement of the left DLPFC in generating context-based priors.

5. Limitations

One of the possible limitations inherent to the present study resides in the cTBS protocol, which aimed at testing modulatory effects on non-motor areas using a fixed stimulation intensity. Indeed, choosing stimulation intensity is not a trivial issue due to the uncertain of the transferability of the motor threshold to other non-motor areas. Furthermore, recent studies have shown the existence of a high inter- and intra-individual variability in M1 responses following cTBS (Hamada, Murase,

Hasan, Balaratnam, & Rothwell, 2013; Jannati, Block, Oberman, Rotenberg, & Pascual-Leone, 2017; Vallence et al., 2015; Vernet et al., 2014). Within this context, the use of fixed or individual stimulation intensity according to participants' rMT remains controversial (Kaminski, Korb, Villringer, & Ott, 2011). Here, based on previous studies that have found cortical modulations in non-motor areas elicited with a 40% of rMT (Kaminski et al., 2011; Komssi & Kahkonen, 2006), and on the fact that participant's rMT were overall relatively low (mean of 45.8%, SD = 4.6%), we decided to apply the safest option according to repetitive transcranial magnetic stimulation guidelines (Wassermann, 1998), which suggest the use of the rMT of the participant with the lowest threshold (please see Kaminski et al. 2011). While we acknowledge that this intensity could have been low, since most consistent responses across subjects has been shown with a TMS intensity of 150% rMT (Vallence et al., 2015), we still where able to observe reliable modulations of cTBS.

6. Conclusions

In the present study, we tested the causal involvement of two brain nodes (beyond the AON) in the recognition of context-embedded actions. Overall, we found that both areas are involved in action prediction; however, their specific contribution to this process differs. More specifically, our results suggest that while the pMTG might specify to the AON which type of grasping movement is more likely to be expected based on knowledge about how objects need to be appropriately grasped for successful action coding, the DLPFC seems to exert a key role in generating and passing high-level context-based priors about the overarching action goal to the AON. These findings suggest that, at least when considering actions embedded in naturalistic contextual settings, motor responses are prone to top-down modulations (Barchiesi & Cattaneo, 2013; Liuzza, Candidi, Sforza, & Aglioti, 2015; Ubaldi et al., 2015). Our design does not allow us to determine whether pMTG and DLPFC are functionally connected and work together in building up priors to facilitate action recognition or are part of parallel distinct pathways (Amoruso, Couto, & Ibanez, 2011). Future studies using

connectivity measures are clearly needed in order to disentangle the specific mechanisms

subserving these processes.

6. References

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Table captions

Table 1. Participants' performance in action recognition. Values corresponding to d' (mean ± SEM) for each context and time-point within the vertex, DLPFC and pMTG sessions.

Table 2. Mean raw MEP amplitudes for each experimental condition. Values corresponding to the raw amplitudes (mean ± SEM) of MEPs recorded from the FDI and the ECR muscles in the 3 observation conditions and baseline trials for the time-points of interest (300ms and 500ms) in the 3 sessions (vertex, DLPFC and pMTG).

Figure captions

Figure 1. Examples of stimuli and conditions. Depending on the action, the reach-to-grasp movement kinematics was different in terms of precision vs. whole-hand grips. In addition, the actions could be performed in three different contexts: congruent, incongruent, and ambiguous.

Figure 2. Study design. The experiment included 3 "measure" single-pulse TMS blocks in which MEPs were recorded from the FDI and ECR muscles. Each block was preceded by an offline "perturb session", where continuous theta burst stimulation (cTBS) was administered over the left pMTG, left DLPFC or over vertex (active control site). The 3 sessions were separated by 60min and their order was counterbalanced across participants. During the "measure" blocks, participants watched occluded videos depicting everyday actions and predicted which action the model was going to perform. In order to build-up their predictions, participants were requested to pay attention to the kinematic information of the model's hand movements and the contextual information in which these movements were embedded. Each trial began with a frame with the word "attention" on the screen during 5000ms, followed by the video-clip, which lasted 500ms. After the video-clip, a frame with the verbal descriptors of two possible end-goals (e.g., 'to drink', 'to clean', one located up and the other located down) was presented. This frame remained on the screen until a response was recorded. TMS pulses were randomly delivered at 300ms or 500ms after video onset.

Figure 3. Behavioural results. Participants' performance in predicting the course of the observed actions (expressed as *d'*) during the 3 action observation conditions (Congruent, Incongruent, and Ambiguous) at the 2 time-windows (early, late) in the 3 Sessions (Vertex, DLPFC, pMTG). Asterisks indicate significant comparison (p<0.05). Error bars represent SEM.

Figure 4. MEPs results. Amplitudes of MEPs recorded from the FDI (A) and ECR (B) muscles during the 3 action observation conditions (Congruent, Incongruent, and Ambiguous) at the 2 time-windows (early, late) in the 3 Sessions (Vertex, DLPFC, pMTG). Asterisks indicate significant comparison (p<0.05). Error bars represent SEM.

Tables

		Vertex	DLPFC	pMTG
		d'	*d'*	*d'*
300ms	Ambiguous	0.442±0.11	0.576±0.09	0.499±0.1
	Congruent	1.417±0.06	1.222±0.08	1.332±0.1
	Incongruent	-0.517±0.18	-0.573±0.15	-0.503±0.17
500ms	Ambiguous	0.471±0.11	0.537±0.11	0.508±0.1
	Congruent	1.339±0.07	1.415±0.07	1.347±0.07
	Incongruent	-0.441±0.2	-0.654±0.2	-0.415±0.2

		Vertex		DLPFC		pMTG	
		FDI	ECR	FDI	ECR	FDI	ECR
300ms	Ambiguous	1.129±0.169	0.551±0.066	1.060±0.163	0.529±0.07	1.077±0.189	0.532±0.072
	Congruent	1.201±0.184	0.502±0.054	1.086±0.175	0.551±0.07	1.195±0.226	0.531±0.079
	Incongruent	1.104±0.159	0.541±0.07	1.110±0.192	0.536±0.073	1.176±0.224	0.540±0.075
500ms	Ambiguous	0.970±0.127	0.493±0.059	1.021±0.157	0.511±0.067	1.007±0.194	0.523±0.075
	Congruent	1.126±0.185	0.506±0.06	1.000±0.163	0.524±0.072	1.126±0.221	0.515±0.065
	Incongruent	0.860±0.123	0.491±0.06	1.003±0.148	0.508±0.074	1.114±0.213	0.471±0.066
	Baseline	0.425±0.042	0.387±0.045	0.438±0.057	0.388±0.054	0.445±0.059	0.381±0.047

Figures

A)

Early time window (300 ms) — Sensitivity d'

Legend:
- Ambiguous
- Congruent
- Incongruent

Vertex   DLPFC   pMTG

Late time window (500 ms) — Sensitivity d'

Vertex   DLPFC   pMTG